Recurrence Phenomena After Immunoglobulin Therapy for Snake Envenomations: Part 1. Pharmacokinetics and Pharmacodynamics of Immunoglobulin Antivenoms and Related Antibodies

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The production of immunoglobulin antivenoms has evolved over the past 50 years, resulting in a choice of source animals and highly purified, target-specific immunoglobulin fragments (IgG, Fab_2, and Fab). Differences in pharmacokinetic and pharmacodynamic properties of these fragments may affect clinical efficacy. For example, both local and systemic recurrences (worsening after initial improvement) with intact or fragmented immunoglobulin antivenoms have been observed. Local recurrence may result in greater tissue injury, and coagulopathic recurrence may result in the risk of hemorrhage. The latter is of particular concern because coagulopathic recurrence usually occurs after patient discharge. Similar phenomena of symptom recurrence have been observed with ovine, digoxin-specific Fab, and with Fab_2 and IgG antivenoms from a variety of source animals as well. Recurrence of venom effects in Fab-treated patients appears to be the result of a pharmacokinetic and pharmacodynamic mismatch between the antivenom and target venom components. That is, tissue penetration and venom neutralization is incomplete, and clearance of unbound antivenom (antivenom that has not bound its venom target) is significantly faster than the clearance of some venom components, allowing signs and symptoms of envenomation to recur. Understanding the relative kinetics and dynamics of immunoglobulins and their targets may allow the physician to anticipate their clinical implications and may suggest modifications of the drug or dose to produce better clinical results.

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INTRODUCTION

Immunoglobulin antivenoms have been in clinical use for many decades. The pharmacokinetics (changes of drug concentration in the body with respect to absorption, distribution, metabolism, and excretion) and pharmacodynamics (the interaction of the drug with biologic receptors and mechanisms of action as the basis for therapeutic or toxic effects) of these agents in relation to the kinetics and dynamics of their target substances determine their clinical efficacy.

The original immunoglobulin products were minimally purified animal sera containing whole IgG, usually of equine (horse) origin. More recently, specific antibody fragments of IgG—Fab and Fab_2—produced by enzyme digestion of IgG, have been produced for a variety of venoms, toxins, and toxicants, and from a variety of animals. Fab and Fab_2 fragments are of smaller molecular weight and are not as immunogenic, with the potential advantages of decreased immediate and delayed hypersensitivity reactions and increased interstitial tissue penetration.[1,2]

Local and coagulopathic recurrence (worsening after initial improvement following immunoglobulin therapy) has been observed during clinical trials with an affinity-purified, mixed monospecific crotaline antivenom, ovine (sheep) Fab for injection (FabAV [CroFab, Protherics]).[3-6] Similar phenomena have been observed with other Fab antivenoms, digoxin-specific Fab, and with Fab_2 and IgG antivenoms as well.[7-9] The cause of this phenomenon appears to be differences between the kinetics and dynamics of the immunoglobulin and its target.[3,10] In the case of FabAV, the clearance of unbound antivenom is significantly faster than the absorption and clearance of some venom components, allowing signs and symptoms to recur. An understanding of the kinetics and dynamics of immunoglobulins and their targets allows assessment of their clinical implications and may suggest modifications of immunoglobulin structure and dose regimens to optimize clinical results.

GENERAL KINETICS AND DYNAMICS OF IMMUNOGLOBULINS

Every immunoglobulin has relatively unique kinetics and dynamics based on its class (eg, IgG, Fab_2, Fab), source animal, valence (monovalent, mixed monovalent, or polyvalent), route of administration, and the specific target toxin or toxicant. Also, individuals may display variable kinetics and dynamics with any particular immunoglobulin, depending on physiologic parameters such as renal function (Table).

Immunoglobulin kinetic data generally fit a 2-compartment model, with a biexponential curve on a log-linear plot.[11] In general, the larger the molecule, the smaller the volume it distributes into and the longer the distribution time and elimination half-life ($t_{1/2\beta}$).[12] Disposition of the toxin/immunoglobulin complex is dependent on the size and charge of the complex, with varying degrees of elimination by the kidney and the reticuloendothelial system (RES).[7,13]

Volume of distribution (Vd) is most dependent on molecular weight. In general, IgG distributes to a volume close to the vascular volume (~90 mL/kg). The smaller Fab_2 and Fab fragments distribute into a volume closer to the extracellular volume (~200 to 400 mL/kg).[8,12-14]

The initial distribution phase of immunoglobulins after intravenous injection is primarily dependent on molecular weight, with larger molecules having slower distributive times. Fab distributes more rapidly and produces a higher interstitial/plasma concentration ratio than IgG or Fab_2. IgG and Fab_2 have similar distribution times and concentration ratios.[12,15]

Immunoglobulin elimination, particularly in relation to target (toxin) elimination, is responsible for a large part of clinical response. The $t_{1/2\beta}$, the time required to reduce the blood concentration by half during the elimination phase, is largely size-dependent, with smaller molecules having faster elimination.[14-17] The larger molecules, IgG and Fab_2, are eliminated in large part by nonrenal processes. Fab, a protein of less than 70,000 daltons, is more likely to be eliminated renally.[18] The proximal tubules are also a site of protein catabolism.[16,19] Charge and source/recipient factors, however, also affect the routes of elimination of the various immunoglobulins.[20-22] For example, renal clearance of a digoxin-specific ovine Fab in human beings was 56%.[13] In contrast, IgG had 72.8% gut and 20.5% liver catabolism in the same model.[12]

Total body clearance (CL_B, the amount of drug removed per unit time by all pathways) is also generally size-dependent but is not solely a function of $t_{1/2\beta}$. In 26 human beings envenomated by the Malayan pit viper, even though the mean $t_{1/2\beta}$ of equine Fab_2 was slightly longer than equine IgG (96 versus 82 hours, respectively) and more than twice as long as caprine (goat) IgG (45.5 hours), clearance of the equine Fab_2 was higher than the caprine IgG (1.67 versus 1.3 mL/h per kilogram), and almost 3 times the clearance of the equine IgG (0.63 mL/h per kilogram).[8]

IgG has a typically long half-life in human beings. The crotaline snake antivenom currently available in the United States, Antivenin (Crotalidae) Polyvalent (Wyeth-Ayerst), has a half-life of 61 to 194 hours in envenomated patients.[23,24] It has been detected in the urine up to 4 months after treatment.[25] The half-life of ovine Fab in human beings is typically much shorter. In a case series of digoxin-toxic patients, the $t_{1/2\beta}$ of ovine, digoxin-specific Fab was 14.3 to 25.4 hours.[13]

Immunoglobulin kinetics can be affected by interactions with their target substances. Venom components are frequently larger than 20,000 daltons and can range up to 130,000 daltons.[26] The Fab/venom complex is too large for renal elimination and therefore remains in circulation longer than free Fab (Fab that has not bound to its target). If there is only partial neutralization of target molecules or if immunoglobulin/target dissociation occurs, this may result in increased or recurrent toxicity.

Aside from basic kinetics, the efficacy of immunoglobulins is a function of their specificity and affinity for venom components, molecular stability, the possible presence of anti-idiotypic antibodies (antibodies produced by the recipient against the therapeutic immunoglobulin), and distribution and concentration at the sites of action. Immunoglobulins generally are of high specificity and usually have significantly higher affinity for their targets than their targets have for body tissues.[7] In other words, immunoglobulins bind their target and usually neutralize their activity.[7,27]

Immunoglobulin antibodies appear to maintain the ability to bind their targets as long as they are in circulation.[7] However, Fab_2 antibodies may break apart at the hinge region, affecting kinetics and dynamics. The degree of cross-linking around the hinge region is a key component in conferring longer half-life and binding affinity and may be artificially manipulated during Fab_2 preparation.[28]

Table.
Immunoglobulin pharmacokinetics in human beings after intravenous administration.

Immunoglobulin Type	Valence	Target	Source	Vd_{ss} (Vd_β)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	Clearance (mL/min)	Notes
IgG	**Polyvalent**	***Crotalus/Agkistrodon***	**Horse**			**158.4**		**Single patient with *C atrox* envenomation[24]**
IgG	Monovalent	*C rhodostoma*	Horse	90 mL/kg	0.46	82	0.525	7 patients; unpurified horse serum[8]
IgG	Monovalent	*C rhodostoma*	Goat	92.5 mL/kg	1.96	45.5	1.118	6 patients; processing to minimize aggregate formation[46]
Fab_2	Monovalent	*C rhodostoma*	Horse	233 mL/kg	0.3	96	1.279	5 patients; pepsin-digested serum[8]
Fab_2	Polyvalent	*Echis*	Horse			18		17 patients with *Echis ocellatus* envenomation[10]
Fab	**Mixed monovalent**	***Crotalus/Agkistrodon***	**Sheep**	**110 mL/kg**	**2.7**	**18**	**5.7**	**4 patients with *Crotalus* snakebite[34]**
Fab	Monovalent	*Echis ocellatus*	Sheep			4.3		22 patients with *E ocellatus* envenomation[10]
Fab	Monovalent	Digoxin	Sheep	10.8 L		12.1	23.4	1 patient with digoxin toxicity[47]
Fab	Monovalent	Digoxin	Sheep	430 mL/kg (Vd_β)		14	0.324 mL/min/kg	7 patients with digoxin toxicity and normal renal function (creatinine clearance=103[13])
Fab	Monovalent	Digoxin	Sheep	369 mL/kg (Vd_β)		9.3	0.389 mL/min/kg	4 patients with digoxin toxicity and impaired renal function (creatinine clearance=38[13])
Fab	Monovalent	Digoxin	Sheep	669 mL/kg (Vd_β)		23.9	7.1	Treatment of digoxin toxicity in patient with serum creatinine=1.4[48]
Fab	Monovalent	Digoxin	Sheep	2,751 mL/kg (Vd_β)		56.3	6.3	Treatment of digoxin toxicity in patient with serum creatinine=1.8[48]
Fab	Monovalent	Digoxin	Sheep	95 mL/kg (Vd_β)		71.8	2.6	Treatment of digoxin toxicity in patient with serum creatinine=4[48]
Fab	Monovalent	Digoxin	Sheep	163 mL/kg (Vd_β)		45.6	2.3	Treatment of digoxin toxicity in patient receiving hemodialysis[48]
Fab	Monovalent	Digoxin	Sheep	290 mL/kg		82	0.049	4 patients with digoxin toxicity, end-stage renal disease, and hemodialysis[7]
Fab	Monovalent	Digoxin	Sheep	36.2 L	49.9	138.6	3.02	1 patient with digoxin toxicity, end-stage renal disease (creatinine clearance 8 mL/min), and peritoneal dialysis[49]

Antivenin (Crotalidae) Polyvalent (IgG) and CroFab (Fab) shown in bold type.
VD_{ss}, Steady-state volume of distribution; $t_{1/2\alpha}$, distribution half-life; $t_{1/2\beta}$, terminal elimination half-life; **polyvalent**, immunogen contains multiple chemicals (eg, venom components); **monovalent**, immunogen contains only one chemical target (eg, digoxin).

The increased penetration of Fab and Fab_2 into the interstitial space, compared with IgG, suggests that such fragments would be better able to neutralize venom, toxins, or toxicants deposited there.[12,14] Unfortunately, this property does not appear to confer enhanced efficacy in treating local venom effects in snakebite envenomations. In vitro, IgG, Fab_2, and Fab are similarly effective in neutralizing locally active venom components. Fab and Fab_2 antibody fragments to *Vipera berus* (European viper) were equal in neutralizing the hemorrhagic tissue effects of the venom in mice.[29] Equine IgG and Fab_2 against *Bothrops asper* (barba amarilla) were protective in mice when mixed with venom before injection, but were only partially and equally effective in neutralizing local tissue damage when injected at different sites from the venom. Even when the antivenom was given before injecting the venom, neither agent was capable of neutralizing all of the venom at the bite site. Although venom-induced capillary disruption may result in extravasation of immunoglobulin into the injured tissue, most neutralization appears to take place at the leading edge of tissue injury and in the central circulation.[30]

In contrast to local effects, antivenom administered 5 or 120 minutes before envenomation completely prevented coagulopathic effects,[30] suggesting that coagulopathic toxicity occurs, and can be prevented, in the central circulation. Thus, all intravenously administered antivenoms should potentially be capable of initial neutralization of coagulopathic venom components.

A closely analogous situation occurs in patients treated with a digoxin-specific Fab, who exhibit a similar recurrence phenomenon to that of antivenoms. Free digoxin concentrations fall rapidly after Fab administration, but then rebound upward in patients with normal renal function within 12 to 24 hours.[7] In patients with renal failure, that rebound is delayed 12 to 130 hours.[7] The possible reasons for the rebound include redistribution of unbound digoxin from tissue stores after unbound antibody elimination, loss of binding ability of the antibody, and dissociation of the Fab/digoxin complex. Dissociation between Fab and digoxin is an unlikely explanation because the binding affinity makes the expected rate of dissociation too small to explain the rebound. Also, the free fraction of digoxin in the presence of Fab remains less than the free fraction in the absence of Fab, indicating continuous binding as long as antibody is present. The most likely explanation in patients with normal renal function is that unbound Fab is eliminated shortly after administration. If free digoxin then redistributed into the central circulation, there would be no Fab available to bind it and free digoxin levels would rebound. Because unbound Fab excretion is impaired in renal failure, a delay to rebound would be expected, as free Fab would still be present to bind redistributing unbound digoxin as it reentered the blood. Rebound of digoxin levels would only occur if and when the excess Fab was completely occupied.

Finally, anti-idiotypic antibodies may develop in the immunoglobulin recipient. An anti-idiotypic antibody forms against the therapeutic antibody, thereby preventing the therapeutic antibody from binding its target molecule. Development of anti-idiotypic antibodies usually requires 1 to several weeks and thus they are unlikely to play a role in the recurrence phenomenon seen in response to a first exposure to an antibody.[31] However, they may cause decreased clinical efficacy in subsequent antibody use.[1]

In preliminary work, unbound FabAV administered during a clinical trial of crotaline envenomation had a $t_{1/2\alpha}$ of 2.5 to 2.7 hours, $t_{1/2\beta}$ of 18 hours, CL_B of 4.9 mL/h per kilogram, and Vd of 110 mL/kg, consistent with other ovine Fab preparations (Table).[32-34]

VENOM KINETICS AND DYNAMICS

Snake venom is made up of dozens of components of varying molecular weights and activities.[35,36] No systematic study of venom component kinetics and dynamics has been undertaken. However, in case reports and case series of snake envenomation, patients exhibit prolonged coagulopathic manifestations, with coagulopathies persisting up to 2 weeks after envenomation, suggesting that coagulopathic components of snake venom remain present and active in the body during this time.[37-42] A case of envenomation by *Crotalus atrox* (Western diamondback rattlesnake) was reported in which venom antigen was detectable in urine at least 4 to 6 days after presentation.[21] Thrombocytopenia has persisted until at least day 10 in a timber rattlesnake envenomation,[38] for 7 to 14 days after envenomation by *Crotalus horridus horridus* (timber rattlesnake),[41] for 8 days after a *Crotalus ruber ruber* (red diamond rattlesnake) bite,[39] and for at least 6 days after envenomations by *Crotalus viridis helleri* (southern Pacific rattlesnake).[42] A case of a *C atrox* envenomation treated without antivenom had persistently abnormal prothrombin time, thrombin time, and reptilase time 8 days after the bite.[40] In most of these reports, the coagulopathy was still present when coagulation monitoring was discontinued.

Venom distribution and activity are altered by antivenom administration. An equine Fab_2 against *Vipera* sp

administered to human beings resulted in a 2.3-fold elevation of the plasma venom concentration and a decline in the $t_{1/2\beta}$ of the venom from 31.92 hours to 16.73 hours compared with the venom alone.[43] At the same time, all unbound venom in the central circulation becomes bound to the antibody.[3,8,29] Thus, antivenom depletes unbound venom in the body and increases total body clearance of venom.

Despite the early use of antivenom, it appears that a depot of unneutralized venom often remains in the body, and therefore prolonged absorption of venom components may occur. The clearance of initially unbound FabAV is much more rapid than that of unbound venom, creating the conditions for recurrent venom antigenemia and its effects (Figure 1). Likewise, recurrent venom antigenemia and its effects may be seen in patients treated with IgG,[7] most likely from an eventual exhaustion of available unbound IgG with unneutralized venom continuing to enter the central circulation (Figure 2).

IMMUNOGLOBULIN STRUCTURAL OR DOSE REGIMEN MODIFICATIONS

Modifications in antivenom production, structure, or dose regimens might improve the venom/antivenom kinetic mismatch and prevent rebound of serum toxicant levels or recurrent venom effects. For example, a second dose of digoxin-specific Fab 7 hours after the initial dose resulted in an increase in bound Fab from 47.6 to 70.2%.[13] In the FabAV clinical trials, administration of scheduled doses of FabAV during the first 18 hours after the initial dose prevented recurrence of local symptoms.[33] Also, it has been proposed that it is theoretically possible to maintain a steady-state blood immunoglobulin concentration that is protective against late recurrence of coagulopathy.[34] Intramuscular injection would likely extend the apparent $t_{1/2\beta}$. Equine Fab_2 $t_{1/2\beta}$ increased 25% after intramuscular injection. However, it also resulted in a maximum plasma concentration (C_{max}) that was only

Figure 1.
Idealized graph (based on preliminary kinetic data[35]) of plasma concentrations of unbound Fab crotaline antivenom (FabAV, diamonds), and unbound venom antigens (triangles) versus time. The administration of 12 vials of FabAV (star) at time 0 results in immediate disappearance of unbound venom from the circulation. Redosing of 2 vials of FabAV at 6, 12, and 18 hours after initial control (solid arrows) prevents local recurrence. Venom antigenemia is not required for local recurrence. When unbound antivenom concentrations fall below some critical level, venom antigenemia may recur, with recurrence of venom coagulopathic effects. Redosing of 2 vials of FabAV at this time (open arrow) may again result in immediate disappearance of unbound venom from circulation.

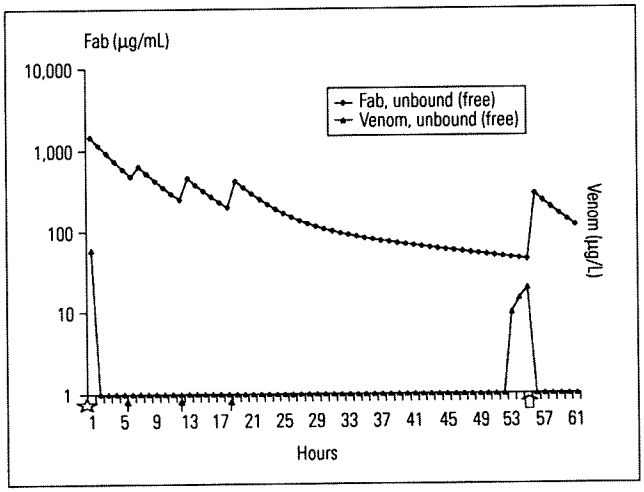
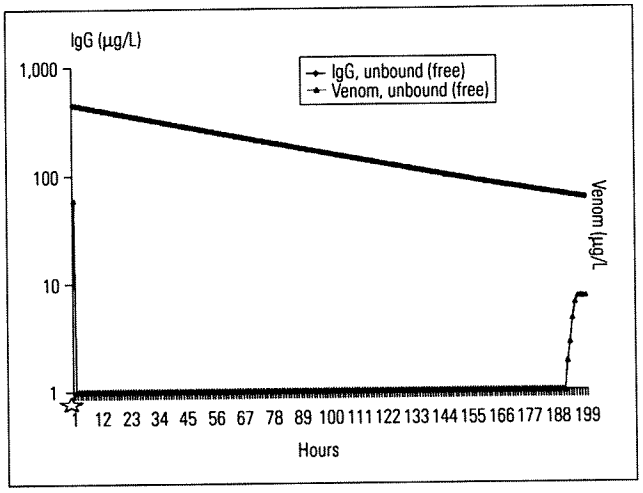


Figure 2.
Idealized graph (based on published kinetic data[23,24]) of plasma concentrations of unbound IgG crotaline antivenom (diamonds) and unbound venom antigens (triangles) versus time. The administration of 10 vials of IgG antivenom (star) at time 0 results in immediate disappearance of unbound venom from the circulation. When antivenom concentrations fall below some critical level, venom antigenemia may recur, with recurrence of venom coagulopathic effects. Typically, this occurs at a later time than with FabAV (190 hours versus 52 hours) because unbound IgG remains in circulation longer to bind venom components entering the central circulation (assuming an appropriate dose of antivenom was administered).



10% of the intravenous dose, a significantly prolonged time to maximal plasma concentration, and less than half fractional drug absorption (f=0.42).[14] Although the slow absorption rate, incomplete absorption, and low C_{max} would make this route a poor choice for initial management, intramuscular use after initial control, perhaps in sustained-absorption vehicles, may provide persistent plasma levels of antivenom sufficient to prevent recurrent coagulopathy.[34]

Other modifications might include improved purification, which increased specific binding activity by 51%[26]; alteration of charge or complexation with glycosate[44] or dextran, which altered biodistribution, kinetics, and catabolism[45]; and coadministration of other substances such as basic amino acids, lysine, or arginine, which have been shown to block renal accumulation of Fab.[44]

In summary, the pharmacokinetics and pharmacodynamics of immunoglobulins determine their clinical efficacy. Every therapeutic immunoglobulin preparation will have relatively unique kinetics and dynamics, but recurrence phenomena seen with immunoglobulin therapy are a result of differences between the kinetics and dynamics of these agents and their target toxins or toxicants. A great disparity between venom and antivenom kinetics and dynamics occurs with Fab antivenoms. The failure to neutralize all venom initially and the more rapid clearance of unbound Fab in relation to venom components may result in recurrence of local and systemic venom effects. Modification of Fab structure or dose regimen may produce a better kinetic and dynamic match between an immunoglobulin and its target.

REFERENCES

1. Smith TW, Lloyd BI, Spicer N, et al. Immunogenicity and kinetics of distribution and elimination of sheep digoxin-specific IgG and Fab fragments in the rabbit and baboon. *Clin Exp Immunol.* 1979;36:384-396.

2. Smith DC, Reddi KR, Laing G, et al. An affinity purified ovine antivenom for the treatment of *Vipera berus* envenoming. *Toxicon.* 1992;30:865-871.

3. Seifert SA, Boyer LV, Dart RC, et al. Relationship of venom effects to venom antigen and antivenom serum concentrations in a patient with *Crotalus atrox* enevenomation treated with a Fab antivenom. *Ann Emerg Med.* 1997;30:49-53.

4. Seifert SA, Dart RC, Bogdan GM, et al. Recurrence of local symptoms of crotalid envenomation in ovine Fab antivenom treated patients [abstract]. *J Toxicol Clin Toxicol.* 1999;37:390.

5. Seifert SA, Dart RC, Boyer LV, et al. Prevention and Treatment of recurrent local symptoms in crotalid envenomation treated with ovine Fab [abstract]. *J Toxicol Clin Toxicol.* 1999;37:668.

6. Boyer LV, Seifert SA, Clark RF, et al. Recurrent and persistent coagulopathy following pit viper envenomation. *Arch Intern Med.* 1999;159:706-710.

7. Ujhelyi MR, Robert S. Pharmacokinetic aspects of digoxin-specific Fab therapy in the management of digitalis toxicity. *Clin Pharmacokinet.* 1995;28:483-493.

8. Ho M, Silamut K, White N, et al. Pharmacokinetics of three commercial antivenoms in patients envenomed by the malayan pit viper, *Calloselasma rhodostoma,* in Thailand. *Am J Trop Med Hyg.* 1990;42:260-266.

9. Hardy DL, Monette J, Corrigan JJ. Envenomation by the northern blacktail rattlesnake *(Crotalus molossus molossus):* report of two cases and the in vitro effects of the venom on fibrinolysis and platelet aggregation. *Toxicon.* 1982;20:487-493.

10. Meyer WP, Habib AG, Onayade AA, et al. First clinical experiences with a new ovine Fab′ *Echis ocellatus* snake bite antivenom in Nigeria: randomized comparative trial with Institute Pasteur serum (IPSER) Africa antivenom. *Am J Trop Med Hyg.* 1997;56:291-300.

11. Azuma J, Kurimoto T, Tsuji S, et al. Phase I study on human monoclonal antibody against cytomegalovirus: pharmacokinetics and immunogenicity. *J Immunother.* 1991;10:278-285.

12. Covell DG, Barbet J, Holton OD, et al. Pharmacokinetics of monoclonal immunoglobulin G1, F(ab′)2, and Fab′ in mice. *Cancer Res.* 1986;46:3969-3978.

13. Schaumann W, Kaufmann B, Neubert P, et al. Kinetics of the Fab fragments of digoxin antibodies and of bound digoxin in patients with severe digoxin intoxication. *Eur J Clin Pharmacol.* 1986;30:527-533.

14. Pepin S, Lutsch C, Grandgeorge M, et al. Snake F(ab′)2 antivenom from hyperimmunized horse: pharmacokinetics following intravenous and intramuscular administrations in rabbits. *Pharm Res.* 1995;12:1470-1473.

15. Lloyd BL, Smith TW. Contrasting rates of reversal of digoxin toxicity by digoxin-specific IgG and Fab′ fragments. *Circulation.* 1978;58:280-283.

16. Wochner RD, Strober W, Waldman TA. The role of the kidney in the catabolism of Bence-Jones proteins and immunoglobin fragments. *J Exp Med.* 1967;126:207-221.

17. Bazin-Redureau MI, Renard CB, Scherrmann J-MG. Pharmacokinetics of heterologous and homologous immunoglobulin G, F(ab′)2 and Fab after intravenous administration in the rat. *J Pharm Pharmacol.* 1997;49:277-281.

18. Chang RS, Ueki IF, Troy JL, et al. Permselectivity of the glomerular capillary wall to macromolecules. II. Experimental studies in rat using neutral dextran. *Biophys J.* 1975;15:887-906.

19. Arend WP, Silverblatt FJ. Serum disappearance and catabolism of homologous immunoglobulin fragments in rats. *Clin Exp Immunol.* 1975;22:502-513.

20. Pentel PR, Keyler DE, Gilbertson DG, et al. Pharmacokinetics and toxicity of high doses of antibody Fab fragments in rats. *Drug Metab Dispos.* 1988;16:141-145.

21. Rennke HG, Patel Y, Venkatachalam MA. Glomerular filtration of proteins: clearance of anionic, neutral, and cationic horseradish peroxidase in the rat. *Kidney Int.* 1978;13:278-288.

22. Bertolatus JA, Hunsicker LG. Glomerular sieving of anionic and neutral bovine albumins in proteinuric rats. *Kidney Int.* 1985;28:467-476.

23. Gillissen A, Theakston RDG, Barth J, et al. Neurotoxicity haemostatic disturbances and haemolytic anaemia after a bite by a Tunisian saw-scaled or carpet viper (*Echis 'pyramidum'*-complex): failure of antivenom treatment. *Toxicon.* 1994;32:937-944.

24. Sorensen HNH, Faber V, Svehag SE. Circulating immune complexes, complement activation kinetics and serum sickness following treatment with heterologous anti-snake venom globulin. *Scand J Immunol.* 1978;7:25-33.

25. Ownby CL, Reisbeck SL, Allen R. Levels of therapeutic antivenin and venom in a human snakebite victim. *South Med J.* 1996;89:803-806.

26. Iwanaga S, Suzuki T. Enzymes in snake venom. In: Lee C-Y, ed. *Snake Venoms.* Berlin: Springer Verlag; 1979:64-65.

27. Owens SM, Mayersohn M. Phencyclidine-specific Fab fragments alter phencyclidine disposition in dogs. *Drug Metab.* 1986;14:52-58.

28. Humphreys DP, Vetterlein OM, Chapman AP, et al. F(ab′)2 molecules made from *Escherichia coli* produced Fab′ with hinge sequences conferring increased serum survival in an animal model. *J Immunol Methods.* 1998;217:1-10.

29. Lomonte B, Leon G, Hanson LA. Similar effectiveness of Fab′ and F(ab′)2 antivenoms in the neutralization of hemorrhagic activity of *Vipera berus* snake venom in mice. *Toxicon.* 1996;34:1197-1202.

30. Gutierrez JM, Leon G, Rojas G, et al. Neutralization of local tissue damage induced by *Bothrops asper* (terciopelo) snake venom. *Toxicon.* 1998;36:1529-1538.

31. Chatenoud L. Humoral immune response against OKT3. *Transplant Proc.* 1993;25(2 Suppl 1):68-73.

32. Dart RC, Seifert SA, Carroll L, et al. Human trial of an affinity purified antibody fragment for snake venom poisoning [abstract]. *Vet Human Toxicol.* 1994;36:363.

33. Seifert SA. Pharmacokinetic analysis of a crotalid Fab antivenom and theoretical considerations for the prevention of coagulopathic recurrence [abstract]. *J Toxicol Clin Toxicol.* 1998;36:526-527.

34. Seifert SA. Pharmacokinetic analysis of a crotalid Fab antivenom and theoretical considerations for the prevention of coagulopathic recurrence. Presented at the North American Congress of Clinical Toxicology, September 15, 1998; Orlando, FL.

35. Bajwa SS, Markland FS, Russell FE. Fibrinolytic enzyme(s) in Western diamondback rattlesnake *(Crotalus atrox)* venom. *Toxicon.* 1980;18:285-290.

36. Ouyang C, Teng C-M, Huang T-F. Characterization of snake venom components acting on blood coagulation and platelet function. *Toxicon.* 1992;30:945-966.

37. Hasiba U, Rosenbach LM, Rockwell D, et al. DIC-like syndrome after envenomation by the snake, *Crotalus horridus horridus. N Engl J Med.* 1975;292:505-507.

38. Furlow TG, Brennan LV. Purpura following timber rattlesnake *(Crotalus horridus horridus)* envenomation. *Cutis.* 1985;35:234-236.

39. Lyons WJ. Profound thrombocytopenia associated with *Crotalus ruber ruber* envenomation: a clinical case. *Toxicon.* 1971;9:237-240.

40. Budzynski AZ, Pandya BV, Rubin RN, et al. Fibrinogenolytic afibrinogenemia after envenomation by Western diamondback rattlesnake *(Crotalus atrox). Blood.* 1984;63:1-14.

41. Tallon RW, Kock KL, Barnes SG, et al. Envenomation coagulopathy from snakebites [letter]. *N Engl J Med.* 1981;305:1347.

42. La Grange E, Russell FE. Blood platelet studies in man and rabbits following Crotalus envenomation. *Proc West Pharm Soc.* 1970;13:99-105.

43. Pepin-Covatta S, Lutsch C, Lang J, et al. Preclinical assessment of immunoreactivity of a new purified equine F(ab')2 against European viper venom. *J Pharmaceut Sci.* 1998;87:221-225.

44. Kobayashi H, Le N, Kim I, et al. The pharmacokinetic characteristic of glycolated humanized anti-Tac Fab' are determine by their isoelectric points. *Cancer Res.* 1999;59:422-430.

45. Fagnani R, Halpern S, Hagan M. Altered pharmacokinetic and tumour localization properties of Fab' fragments of a murine monoclonal anti-CEA antibody by covalent modification with low molecular weight dextran. *Nucl Med Comm.* 1995;16:362-269.

46. Mordenti J, Chen SA, Moore JA, et al. Interspecies scaling of clearance and volume of distribution data for five therapeutic proteins. *Pharmaceut Res.* 1991;8:1351-1359.

47. Thanh-Barthet CV, Urtizberea M, Sabouraud AE, et al. Development of a sensitive radioimmunoassay for Fab fragments: application to Fab pharmacokinetics in humans. *Pharm Res.* 1993;10:692-695.

48. Allen NM, Dunham GD, Sailstad JM, et al. Clinical and pharmacokinetic profiles of digoxin immune Fab in four patients with renal impairment. *DICP.* 1991;25:1315-1320.

49. Caspi O, Zylber-Katz E, Gotsman O, et al. Digoxin intoxication in a patient with end-stage renal disease: efficiency of digoxin-specific Fab antibody fragments and peritoneal dialysis. *Ther Drug Monit.* 1997;19:510-515.